ELISA plates. 33.C9 ( ), 33.H11(O), patient sera LG (⩓) and JP (_), anti-dsDNA(AP) (■), and anti dsDNA(IK) (□) were preincubated with 0, 0.1, 1.0, 10, 100, or 1,000 µg/ml of calf thymus dsDNA (———) or Yeast tRNA (- - -) before being added to the plates.

Figures 2a and 2b are the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of the cDNA insert in G7. Asterisks show the stop codon. This sequence has been added to the GenBank nucleic acid sequence database, Los Alamos National Laboratory, NM, and has been assigned accession number U27517.

Figure 3a and 3b are an alignment of the central core regions of 5 ribosomal proteins (SEQ ID NOS:3 to 7)(r-proteins) S1. Asterisks show the 5 repeating regions (SEQ ID NOS:8 to 12)(a, b, c, d, and e, respectively). Spaces indicate positions where gaps were introduced to optimize alignment of the sequences (SEQ ID NOS:3 to 7). Dashes indicate identity to the residues of HS1 (SEQ ID NO:3). Alignment of the central core region of HS1 (SEQ ID NO:3) is residues 63-317. HS1; human r-protein S1 (SEQ ID NO:3)presented in this study, ES1; E. coli r-protein S1 (SEQ ID NO:4)(Ref. 26), RS1; Rhizobium melilotii r-protein S1 (SEQ ID NO:5)(Ref. 28), PS1, Providencia sp. r-protein S1 (SEQ ID NO:6)(Ref. 27), CS1; chloroplast r-protein S1 (SEQ ID NO:7)(Ref. 29).

Figures 4A-D are graphs comparing the affinity of 33.H11 and 33.C9 for G7-RP or DNA in inhibition ELISA. 33.H11 [A (0.0031 µg/ml) and C (1.0 µg/ml)] and 33.C9 [B (0.32 µg/ml) and D (0.56 µg/ml)] were

preincubated with different concentrations of calf thymus dsDNA ( ), G7-RP (O), Yeast tRNA (▲), or BSA (_), before adding the antibodies to the plates coated with calf thymus dsDNA (A and B) or G7-RP (C and D).

The same result obtained by administering peptide or a peptide conjugate can be achieved by coupling recombinant or isolated human ribosomal protein S1 to human IgG.

Although described herein with reference to the whole protein, it is preferable to use peptides of between a few amino acids up to about 100 amino acids, more preferably less than forty amino acids, still more preferably less than ten to twenty amino acids. These peptides can be easily ascertained by immobilizing the anti-dsDNA antibodies from a patient(s) and screening for binding of the peptides. Peptides can be prepared using standard techniques for amino acid synthesis or recombinantly, by engineering the cDNA (SEQ ID NO:1) encoding the protein, described in Figures 2a and 2b.

Anti-Id Antibodies That Are Immunoeactive With Anti-dsDNA Antibodies.

As demonstrated by Example 3, normal human sera contains anti-Id antibodies immunoreactive with anti-dsDNA antibodies present in many SLE patients. Antibodies for use in treating patients can be obtained using standard techniques to harvest antibodies from normal people, or, more preferably, antibody producing cells are isolated by binding of cells expressing antibody using a method as described in Example 3 for isolation of antibody. The antibody producing cells are then transformed with Eppstein-Barr virus (EBV), amplified in culture, the gene encoding the variable region of the anti-Id antibodies cloned, inserted into an appropriate vector, and expressed in bacteria or another

appropriate expression system, using known techniques. Preliminary studies have yielded several clones.

(not shown). Thus, it appears that this 104 kDa protein at least in MOLT4 cell extract is the responsible protein of which G7 encodes a portion.

Sequence analysis of the cDNA insert in G7

The nucleotide sequence of the cDNA insert in G7 was determined. Its primary nucleotide (SEQ ID NO:1)and deduced amino acid (SEQ ID NO:2) sequences (GenBank no. U27517) are shown in Figures 2a and 2b. The cDNA insert (SEQ ID NO:1) proved to be 1,314 nucleotides in length. The TAA stop codon is located at positions 1057-1059. The predicted molecular weight for the encoded polypeptides (SEQ ID NO:2) (352 amino acids) is 38.0 kDa. However, this cDNA insert (SEQ ID NO:1) in G7 seems to be a partial length cDNA because the molecular weight of the encoded polypeptide (SEQ ID NO:2) is smaller than the estimated full length size (104 kDa) of the reactive protein in MOLT4 cell extract. Thus, this cDNA (SEQ ID NO:1) does not seem to contain the initiation codon.

A search for similarities between the nucleotide sequence of the cDNA (SEQ ID NO:1) in G7 (GenBank no. U27517) and other sequences through the NCBI using the BLAST network service showed a significant match (99% identity) with a sequence encoding human ribosomal protein (r-protein) S1 homologue mRNA reported by Eklund at al., *Gene* 155:231 (1995) (SEQ ID NO:3). However, there are 3 nucleotide and 1 amino acid differences between the G7 cDNA insert(SEQ ID NO:1) and their cDNA sequence (SEQ ID NO:3) (GTC (positions 130-132) in the G7 cDNA

insert (SEQ ID NO:1) vs GTA (positions 292-294) in their cDNA (SEQ ID NO:3), AGT (positions 133-135, encodes Ser at residue 45) in the G7 cDNA insert (SEQ ID NO:1). vs GCT (positions 295-297, encodes Ala at residue 99) in their cDNA (SEQ ID NO:3)]. Moreover, 2 nucleotides (C at positions 1355 and 1366) and 162 nucleotides (positions 1-162) in their cDNA (SEQ ID NO:3) are deleted in the G7 cDNA insert (SEQ ID NO:1). A search was made for some similarities between the

predicted amino acid sequence (SEQ ID NO:2) and other protein sequences in the SWISSPROT database using the algorithm as described by Gish, et al., *Nature Genetics* 3:266 (1993); Altschul, et al., *J. Mol. Biol.* 215:403 (1990).

High degree of homology between the central core region (residues 63-317) of the predicted amino acid sequence (SEQ ID NO:2) of this protein and those of several r-proteins S1.

Identify and similarity with r-proteins S1 are the following; 39% identity and 65% similarity with E. coli r-protein S1 (ES1) (SEQ ID NO:4) (26), 40% identity and 64% similarity with Providencia sp. r-protein S1 (PS1) (SEQ ID NO:6) (Schnier, et al., *Mol. Gen. Genet.* 200:476 (1985)), 38% identity and 63% similarity with Rhizobium melilotti r-protein S1 (RS1) (SEQ ID NO:5) (Schnier, et al., *Nucleic Acids Res.* 16:3075 (1988)), and 50% identity and 71% similarity with chloroplast r-protein S1 (CS1) (SEQ ID NO:7) (Franzetti, et al., *J. Biol. Chem.* 267:19075 (1992)). Moreover, 5 repeating regions [EGTV (SEQ ID NO:8) due 158-161 and 243-246), DFGAFV (SEQ ID NO:9) (166-171 and 251-256), GLVHVS (SEQ ID NO:10) (178-183 and 264-269), GDKV (SEQ ID NO:11) (200-203 and 286-289), and RISLS (SEQ ID NO:12) (216-220 and 302-306)] were observed in the protein sequence (SEQ ID NO:2). These repeating residues (SEQ ID NOS:8 TO 12) have a high degree of homology among other r-proteins S1 (Figures 3a and 3b).

Inhibition immunofluorescence

Inhibition of indirect immunofluorescence was measured using Hep 2 cells. Staining of Hep 2 cells by 33.H11 or 33.C9 without preincubation, after preincubation with G7-RP [100 µg/ml for 33.H11 or 1,000 µg/ml for 33.C9], or after preincubation with calf thymus dsDNA [1,000 µg/ml for 33.H11 or 1.0 µg/ml

affinity for G7-RP than for DNA while 33.C9 has a much higher affinity for DNA than for G7-RP. These differences of affinities correlate with the different staining patterns of IFA exhibited by the 2 monoclonal anti-dsDNA antibodies. In that view, 33.h11 binds the cytoplasm plus nucleolus where one would expect the r-protein S1 to be localized and 33.C9 binds the nucleus where DNA is localized.

Table I. Kd (dissociation constant) (mol/1) of the two human IgG monoclonal anti-dsDNA antibodies for calf thymus dsDNA or G7-RP.

	ligand	
antibodies	calf thymus dsDNA	G7-RP
33.H11	1.0×10^{-7}	6.5×10^{-8}
33.C9	3.0×10^{-9}	3.9×10^{-7}

Discussion

In summary, a lambda gt11 cDNA library constructed from mRNA of human liver was screened by using a SLE patient serum with anti-dsDNA antibody and a clone G7 which has a 1.3-kb cDNA insert (SEQ ID NO:1) isolated. Not only all of the 10 anti-dsDNA patient sera but also affinity-purified anti-dsDNA and human IgG monoclonal anti-dsDNA antibodies recognized the protein expressed by G7. The affinity-purified antibody eluted from this protein was positive for anti-dsDNA antibody activity by the Crithidia assay. Moreover, antibody binding to this protein was inhibited completely by DNA but not by RNA. From those observations, it was concluded that anti-dsDNA

antibodies cross-react with the protein expressed by G7.

A significant match (99% identify) between the nucleotide sequence of the cDNA in G7 (SEQ ID NO:1) and a cDNA (SEQ ID NO:3) reported by Eklund et al. as encoding human r-protein S1 homologue mRNA was found. It appears that anti-dsDNA antibodies directly bind to the protein expressed by G7 because the reactivity of anti-dsDNA antibodies against the protein was not influenced by DNAase I treatment and the binding of anti-dsDNA antibodies to the protein was inhibited completely by DNA. The predicted amino acid sequence (SEQ ID NO:2) presented in this study had homology with some r-proteins S1 including ES1 (SEQ ID NO:4). ES1 (SEQ ID NO:4) is well characterized at the functional and structural level (Subramanian, *Prog. Nucleic Acids Res. Mol. Biol.* 28:101 (1983)) while there are few reports about mammalian r-proteins S1. ES1 (SEQ ID NO:4) is the largest protein of the ribosome and has the same length as the ribosome. This protein is associated with the 30S ribosomal subunit in prokaryotes via its N-terminal globular domain and is known to stimulate translation by facilitating mRNA binding to the 30S ribosomal subunit. The central and C-terminal region contain repeating homologous sequences which are known to play a key role in the binding of structural elements of r-protein S1 to mRNA. Five repeating regions (EGTV (SEQ ID NO:8), DFGAFV (SEQ ID NO:9), GLVHVS (SEQ ID NO:10), GDKV (SEQ ID NO:11) and RISLS (SEQ ID NO:12)) which repeat twice in the central core region of the protein (SEQ ID NO:2) were observed (Figures 3a and 3b). This apparent gene duplication which encodes this repeat region is absent only in the chloroplast r-protein S1.

Cytoplasmic and nucleolar staining patterns in IFA suggests that the antigenic target is the ribosome. This is the IFA pattern of human monoclonal anti-dsDNA antibody 33.H11 which is inhibited by both

100 µg/ml of G7-RP and higher concentrations (1,000 µg/ml) of DNA. These results show that G7 encodes a ribosomal protein which is recognized by anti-dsDNA antibodies. From these observations, it is concluded that G7 encodes a part of human r-protein S1 (HS1).

It has been reported that anti-dsDNA antibodies have high frequencies of basic amino acids carrying positive charges in the heavy chain complementarity determining regions and that arginine is the most versatile amino acid for binding with negative-charged DNA (31). However, there are no high scoring negative charged segments, which could be an epitope for anti-dsDNA antibody, in the primary sequence of HS1. These observations might suggest that cross-reactions between anti-dsDNA antibody and HS1 are not dependent on charge interaction in the primary sequence alone but rather that the cross-reactive epitope depends on conformational apposition of negative charges in the tertiary structure of HS1. However, it is likely that HS1 mimics DNA because anti-dsDNA antibodies cross-react with HS1. It is also appealing to believe that proteins which "mimic" the structure of DNA could play a role as immunogen.

Both 33.H11 and 33.C9 are IgG monoclonal anti-dsDNA antibodies (Winkler, et al., *Clin. Exp. Immunol.* 85:379 (1991)) and strongly recognized the protein expressed by G7. However, in IFA, 33.H11 did not show a homogeneous nuclear pattern and the homogeneous nuclear staining patterns of 33.C9 was not inhibited by G7-RP but was inhibited by as little as 1.0 µg/ml of DNA. Also in ELISA, much lower

concentrations of DNA were able to inhibit the binding of 33.C9 to G7-RP compared with that of 33.H11 while much higher

concentrations of G7-RP were needed to inhibit the binding of 33.C9 to DNA compared with that of 33.H11. Although the binding of 33.H11 (1.0 μg/ml) to G7-RP did not seem to be inhibited even by 1,000 g/ml of DNA (Figure 4C), that of 33.H11 (0.0071 g/ml) to G7-RP were inhibited completely by DNA (Figure 1). Therefore, it is likely that this concentration (1.0 g/ml) of 33.H11 in Figure 4C is too high to be inhibited by DNA. Analysis of K_d value showed that 33.H11 has a higher affinity for HS1 than for DNA while 33.C9 has a higher affinity has a higher affinity for DNA than for HS1. In most anti-dsDNA-positive SLE patient sera, anti-dsDNA antibodies behave like 33.C9 which has a higher affinity for DNA than for HS1 but indeed recognizes HS1. Anti-dsDNA antibodies of this type likely predominate because such sera rarely show a cytoplasmic and nucleolar staining pattern but rather a classical nuclear pattern but rather a classical nuclear pattern in IFA as does 33.C9.

Yanase, et al. *Lab. Invest.* 71:52 (1994), have reported that anti-dsDNA antibodies penetrate living cell membranes and bind to cytoplasmic proteins before binding to the nucleus. From these standpoint, anti-dsDNA antibodies like 33.H11 recognize HS1 strongly and are trapped in the cytoplasm preventing their entry into the nucleus. In studies with living PK15 cells, 33.H11 penetrates the plasma membrane and indeed localizes in the cytoplasm.

IT is assumed that most of the amino acid sequences which G7 encodes are mRNA-binding sites on

HS1 because five repeating regions (SEQ ID NOS:8 to 12) (residues 158-306) which repeat twice in HS1 (SEQ ID NO:3) and are supposed to be a